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THE EFFECT OF AGE ON THE ROLE OF HYDROXYPROLINE IN THE HYDROTHERMAL SHRINKAGE OF COLLAGEN

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The view that the part taken by hydroxyproline in crosslinking in collagen increases with the age of the animal is confirmed by the observation that the hydroxyproline content in the dialysate fraction of hydrothermally shrunk collagen is inversely dependent on age. No free hydroxyproline was found in any fraction of shrunken collagen showing that this amino acid is not liberated in the process of hydrothermal shrinkage of collagen.

It was reported that ageing causes a rise in shrinkage temperature of collagenous tissue[1,2] and it was considered to be due to the formation of intermolecular crosslinks which may be inherent crosslinks such as inherent ester links[1] or links involving metallic or aldehydic[3,4] impurities that are food metabolite intermediates, which accumulate in the body on ageing.[3] The recent preliminary findings of the authors,[5] however did not subscribe to the view that the metallic impurities, getting accumulated in the body, can cause a rise in the shrinkage temperature of collagen. Gustavson[6] while supporting the contention that greater crosslinking of collagen takes place on ageing, observed that the bonds involving hydroxyproline increased with age. Verzar[7,8] was in agreement with Gustavson because of his observation that bovine collagen on shrinkage in Ringers solution at 65°C for a stipulated time of 10 minutes released hydroxyproline and the extent of release was decreased with age. While increased involvement of hydroxyproline in the H-bonding of collagen as the animal ages is possible, the liberation of this amino acid on shrinkage is not likely to take place. On shrinkage, the polypeptide chains in collagen get disoriented. In such a helix coil transition, there may not be any possibility of amino acids, getting liberated, particularly hydroxyproline, on hydrothermal shrinkage of collagen. Experiments were performed to verify the point and the results are presented in this paper.

Experimental

Collagen fibres were excised from the tails of male albino rats of different

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ages immediately after sacrifice. The ages were less than 1 month, 2–3 months, 1 year and 2 years. The fibres were washed first with 0.45 M sodium chloride solution and then with water. About 0.1 g. of each non-dried tendon of approximately the same moisture content was immersed in 2 ml. distilled water at 65°C for 10 minutes, a time which is more than sufficient for complete shrinkage of collagen fibres in water. They were then centrifuged at 8000 r.p.m. to separate solubilised and non-solubilised fractions. As only free amino acids can diffuse through cellophane membrane but not solubilised collagen or gelatin, the solubilised fraction was dialysed in a cellophane tube against 50 ml distilled water, for a period of 48 hours at 4°C. The dialysate as well as the water against which the dialysis was carried out were concentrated. These and the non-solubilised fractions were hydrolysed overnight with 6N HCl for estimating the hydroxyproline by the Neumann and Logan method.[9] The hydroxyproline content of the fractions mentioned above are respectively designated as "dialysate hydroxyproline", "dialysable hydroxyproline" and "residue hydroxyproline" in this article. The percentage hydroxyproline contents of each fraction from the tail tendons of the animals of different ages were compared.

Results and Discussion

It is evident from the results (Table I) that no hydroxyproline is liberated on thermic contraction of collagen of any age group.

In view of the observation of earlier workers that free hydroxyproline is present in animal tissues[10,11,12] it may be said that the liberation of free hydroxyproline observed by Verzar on thermic contraction of collagen is probably due to the presence of hydroxyproline

Table 1

DISTRIBUTION OF HYDROXYPROLINE IN VARIOUS FRACTIONS OF COLLAGEN OF RTT*

Sl. No.	Age of the animal	Dialysable hydroxy-proline %	Dialysate hydroxy-proline %	Residue hydroxy-proline %
1.	Less than 1 month ..	Nil	99	1
2.	1 month ..	Nil	79	21
3.	2 months ..	Nil	69	31
4.	1 year ..	Traces	63	37
5.	2 years ..	Nil	67	33

*Average of 6 experiments

itself in the collagenous tissue. In the present experiment, RTT which is considered to be one of the naturally occurring purest form of collagen was used. Further, it was washed well to remove soluble material adhering to it. This point of view is substantiated by the observation that skins of 1 year old rats when subjected to thermic contraction after removal of adhering material and hair was found to have about 1% dialysate hydroxyproline. Skin, being a three dimentional network, free hydroxyproline present may not be easily removed by washing.

Results given in Table I also indicate that upto about 2 months of age, hydroxyproline content of the dialysate fraction is more than that of the dialysate fraction of tendon of later ages. As the animal matures, the hydroxyproline content of this fraction is decreased while that of the residue is increased. This is in conformity with the view that collagen gets more and more crosslinked with age resulting in decreased solubility of collagen. The observation of Butzow and Eichhorn[13] on acetic acid solubility of collagen is similar to this but they reported little change in solubility bstwen 1 and 3 months and appreciable change between 12 and 24 months age groups. However, the trend in the subunit composition of soluble collagen reported by them is similar to that exhibited by dialysate hydroxyproline in the present work. There is a general decrease in the dialysate hydroxyproline as age increases. That the decrease is rapid in the early age of the animal upto 1 month and is slower at later age can be seen from the table. In general, this and Butzow and Eichhorn's findings do support the view that solubility of collagen decreases with age.

It may be concluded that the hydroxyproline is not liberated free by the hydrothermal shrinkage of collagen. This property of collagen is not age dependent though certain amino acid residues of collagen like hydroxyproline get more and more involved in crosslinking on ageing of a living being.

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